HYDROPEROXIDE-SUPPORTED CYTOCHROME P-450-LINKED FATTY ACID HYDROXYLATION IN LIVER MICROSOMES

Åke ELLIN and Sten ORRENIUS

Department of Biochemistry, University of Stockholm and Department of Forensic Medicine, Karolinska Institutet, S-104 01 Stockholm 60, Sweden

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1. Introduction

The ω-oxidation of fatty acids in mammals has been shown to be catalyzed by the cytochrome *P*-450-linked monooxygenase system in liver as well as kidney microsomes [1,2]. The products formed in both tissues include the ω- and (ω−1)-hydroxy fatty acids [2,3]. Björkhem and co-workers found differences in the formation of the ω-hydroxy and (ω−1)-hydroxy products by liver microsomes [3–5], which were interpreted as due to the involvement of two different cytochrome *P*-450 species in these hydroxylations [4,5]. It was subsequently speculated by Moldéus et al. [6] that of the two enzymes involved, the ω-hydroxylase would be specific for fatty acid hydroxylation while the (ω−1)-hydroxylase might be equivalent to the less specific cytochrome *P*-450 catalyzing the hydroxylation of a great variety of foreign compounds including many drugs and other xenobiotics. This hypothesis was also in line with the findings of Frommer et al. [7] in their study on the hydroxylation of *n*-hexane by liver microsomes.

In contrast, kidney microsomal fatty acid hydroxylation was found by Ellin et al. [8] to most probably be due to the action of only one species of cytochrome *P*-450, cytochrome *P*-450_K [2], catalyzing the formation of both the ω- and the (ω−1)-hydroxy products at a ratio determined by the length of the fatty acid. This cytochrome *P*-450_K-linked monooxygenase system had earlier been shown to be specific towards fatty acid hydroxylation [2,9].

Recently, O'Brien and co-workers [10] demonstrated that liver microsomal cytochrome *P*-450 dependent hydroxylations of aromatic compounds could be supported by cumene hydroperoxide which provided the hydroxylation reaction with both reducing equivalents and oxygen, thus replacing NADPH and molecular oxygen. In this paper we have studied the cumene hydroperoxide-supported fatty acid hydroxylation catalyzed by liver and kidney microsomes as well as partially purified cytochrome *P*-450 and cytochrome *P*-450_K. Results are presented which strongly support the assumption that liver microsomes contain both an ω- and an (ω−1)-hydroxylase, the (ω−1)-hydroxylase being the less substrate specific of the two also catalyzing the hydroxylation of other non-fatty acid compounds. The ω-hydroxylase of liver microsomes is probably closely related to the specific fatty acid hydroxylase of kidney microsomes.

2. Materials and methods

Unstarved, male Sprague-Dawley rats (200 g) were used in this study. Liver and kidney microsomes were isolated as described elsewhere [8,11] and protein was determined according to Lowry et al. [12]. Partially purified cytochrome *P*-450 from liver microsomes and cytochrome *P*-450_K from kidney microsomes, were prepared as described by Lu and Levin [13] and by Moldéus et al. [6], respectively.

NADPH- and O_2-dependent lauric acid hydroxylation was measured after aerobic incubation of microsomes with sodium laurate according to Ellin et al. [2]. When cumene hydroperoxide was used, the pH was set to 8.0 and Mg^{2+}, Mn^{2+}, $NADP^+$, isocitrate and isocitrate dehydrogenase were omitted from the

incubation mixture. The incubations were started by the addition of cumene hydroperoxide (5–30 mM suspension in water) using a microliter syringe. Although the reaction was linear with time for only 5 min, the incubation time was set to 10 min in order to be able to detect also very low activities. The hydroxylated products were assayed using the gas liquid chromatographic method of Björkhem and Danielsson [3], slightly modified by Ellin et al. [8].

Cytochrome *P*-450 was determined as described by Kupfer and Orrenius ([14], cf. [9]).

Lauric acid, $NADP^+$ and isocitrate were obtained from Sigma Chemical Co. (St. Louis, Mo., USA), isocitrate dehydrogenase from Boehringer GMBH (Mannheim, Germany), ^{14}C-labelled lauric acid from The Radiochemical Centre (Amersham, England) and cumene hydroperoxide from E. Merck (Darmstadt, Germany). All other chemicals were standard commercial products obtained from local sources.

3. Results

When liver microsomes were incubated with lauric acid in the presence of cumene hydroperoxide, ($\omega-1$)-hydroxy lauric acid was formed. On the other hand, no detectable amount of ω-hydroxy lauric acid was observed. The pH optimum of the ($\omega-1$)-hydroxylation reaction was about pH 8. As may be seen in fig.1, the rate of ($\omega-1$)-hydroxylation increased with increasing cumene hydroperoxide concentration to about 75 μM after which concentration a sharp decrease in activity occurred. Using a standard incubation mixture containing NADPH, the same liver microsomal preparation catalyzed the formation of both ω- and ($\omega-1$)-hydroxy lauric acid at a ratio of 1:5 and the activity was only slightly higher than with cumene hydroperoxide.

In contrast, kidney microsomes, which were highly active with NADPH as a source of reducing equivalents, produced only very small amounts of ($\omega-1$)-hydroxy lauric acid with cumene hydroperoxide, and no ω-hydroxy lauric acid at all was detected.

Cumene hydroperoxide-supported lauric acid hydroxylation was also assayed using samples of partially purified cytochrome *P*-450 and cytochrome $P\text{-}450_K$ instead of microsomes. The results are presented in fig.2. As with liver microsomes, only ($\omega-1$)-hydroxy lauric acid could be detected after incubation of liver cytochrome *P*-450 with lauric acid. The optimal cumene hydroperoxide concentration was four times higher than with liver microsomes, although

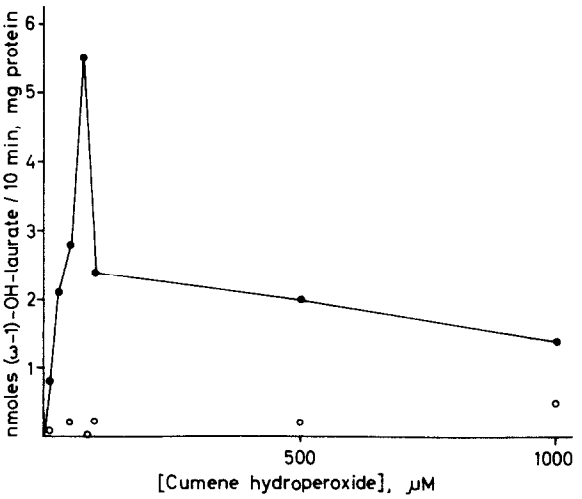

Fig.1. Cumene hydroperoxide-supported hydroxylation of lauric acid catalyzed by liver (•) and kidney (○) microsomes. With liver microsomes protein concentration was 0.44 mg/ml, with kidney microsomes 0.55 mg/ml. *P*-450 concentration was 0.35 nmol/ml and 0.10 nmol/ml respectively. NADPH-supported activities were in nmol/min mg protein with liver microsomes ($\omega-1$)-OH: 0.73; ω-OH: 0.15 and with kidney microsomes ($\omega-1$)-OH: 0.82; ω-OH: 1.13.

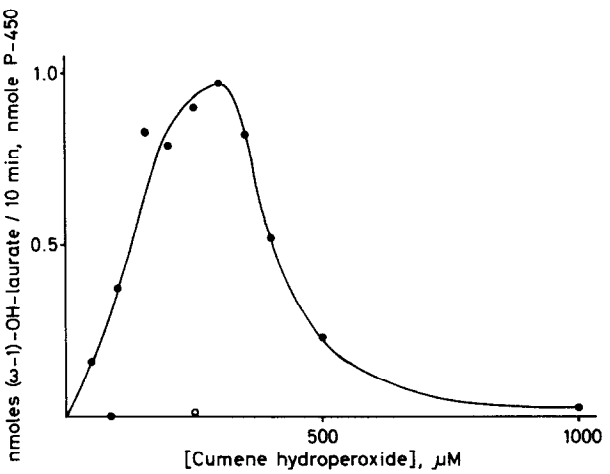

Fig.2. Cumene hydroperoxide-supported lauric acid hydroxylation catalyzed by partially purified liver cytochrome *P*-450 (•) and kidney cytochrome *P*-450 (○). Cytochrome *P*-450 concentration was 2.3 nmol/ml and cytochrome $P\text{-}450_K$ concentration was 0.10 nmol/ml.

the hydroxylation activity per cytochrome *P*-450 was lower than with microsomes. As expected, almost diminishingly small amounts of ($\omega-1$)-hydroxy lauric acid were produced by partially purified cytochrome $P\text{-}450_K$.

4. Discussion

Since previous studies by Ziegler et al. [15] and O'Brien and collaborators [10] had shown that cytochrome *P*-450 can catalyze an organic hydroperoxide dependent hydroxylation of various amines and aromatic compounds, presumably due to a peroxidase-like function of this hemoprotein, we undertook this series of experiments using lauric acid as a substrate with two goals in mind. Firstly, we wanted to investigate whether cytochrome *P*-450 dependent fatty acid hydroxylation can also be supported by the organic hydroperoxide and, secondly, we were interested as to whether different cytochrome *P*-450 species may reveal differences with regards to their interaction with the hydroperoxide. As outlined above, previous work by Björkhem and collaborators [3–5], as well as in our laboratory [6,8] had clearly indicated that different cytochrome *P*-450 species are involved in the ω- and ($\omega-1$)-hydroxylation of fatty acids in liver microsomes, whereas a single cytochrome *P*-450 species (ω-hydroxylase) seems to support the same reactions in kidney microsomes. The present study has given further strong support for this assumption.

Thus, in the presence of cumene hydroperoxide liver microsomes and the partially purified liver microsomal cytochrome *P*-450 preparation catalyzed exclusively the ($\omega-1$)-hydroxylation of lauric acid, whereas the corresponding kidney preparations produced minimal, but significant, amounts of the same product. In no case was the ω-hydroxy derivative formed in the cumene hydroperoxide-supported system. It is also interesting to note that the ($\omega-1$)-hydroxylation activity in liver microsomes reached a sharp maximum at a hydroperoxide concentration of about 75 μM. This is similar to the previous finding with 3,4-benzpyrene as substrate [10] and possibly due to a destruction of cytochrome *P*-450 at higher peroxide concentrations.

Most interesting is the finding that no ω-hydroxy lauric acid was found in the cumene hydroperoxide-supported system suggesting that only the ($\omega-1$)-hydroxylase can interact with the organic hydroperoxide. In contrast to the ω-hydroxylase, the ($\omega-1$)-hydroxylase of liver microsomes has also previously been shown to share other properties with the 'non-specific' drug hydroxylase present in these particles, i.e. inducibility by phenobarbital treatment [3] and inhibition with SKF 525-A and metyrapone [6]. On the other hand, the ω-hydroxylase of liver microsomes behaves similar to the fatty acid hydroxylase (ω- and ($\omega-1$)) of kidney microsomes with respect to lack of response to the same inducer and inhibitors [2,6]. As evident in fig.1, also kidney microsomes seem to contain a low proportion of the 'non-specific' ($\omega-1$)-hydroxylase which, however, would contribute less than 10% to the overall lauric acid hydroxylation activity in this fraction. This would also be in accordance with the finding that rat kidney microsomes show a low but significant hydroxylation activity with some non-fatty acid compounds [9].

In conclusion, this study has provided further evidence that i) two cytochrome *P*-450 species are involved in the ω- and ($\omega-1$)-hydroxylation of fatty acids in liver microsomes, whereas a single cytochrome *P*-450 species (ω-hydroxylase) catalyzes by far the major part of fatty acid hydroxylation in kidney microsomes, ii) that a similar, possibly identical, ω-hydroxylase is present in both liver and kidney microsomes and iii) that this enzyme can catalyze both the ω- and ($\omega-1$)-hydroxylation of fatty acids at a ratio determined by the length of the fatty acid, whereas the ($\omega-1$)-hydroxylase cannot catalyze hydroxylation in the ω-position.

Acknowledgements

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